Isolated photoprotein bolinopsin, and the use thereof

The invention relates to the photoprotein bolinospin, to its nucleotide and amino acid sequences and to the activity and use of the photoprotein bolinopsin.

Photoproteins

The phenomenon of the generation of light by living organisms is designated bioluminescence. It is the result of biochemical reactions in cells, in which reactions the chemical energy is emitted in the form of light quanta (what is termed cold emission by means of chemoluminescence). While the light which is produced in this way is monochromatic, since it is emitted in connection with a discrete electron transfer, it can be shifted by secondary luminescent dyes (e.g. fluorescent proteins in the case of luminescent jellyfish of the genus Aequoria) into spectral regions of longer wavelength.

Bioluminescence has a diversity of biological functions: at an ocean depth of between 200 and 1000 m (mesopelagial), about 90% of all living organisms luminesce. In this case, the luminescent signals are employed for attracting partners, for deception and as a lure. Glowworms and fireflies also use the light signals for seeking partners. On the other hand, the significance of the luminescence of bacteria, fungi and single-cell algae is unclear. It is assumed that it is used for coordinating many single individuals in a large population or else represents a type of biological clock.

A large number of coelenterates are bioluminescent (Morin et al., 1974). These organisms emit blue or green light. As an isolated protein, aequorin, which is derived from Aequoria victoria (Shimomura et al., 1969) and which, in 1962, was the first light-producing protein to be identified, emitted a blue light, and not a green light as observed phenotypically in the case of Aequoria victoria. The green fluorescent protein (GFP) which, as a result of being activated by aequorin, causes Aequoria victoria to appear phenotypically green was subsequently isolated from this medusa (Johnson et al., 1962; Hastings et al., 1969; Inouye et al., 1994). Other photoproteins which have also been identified and described are clytin (Inouye et al., 1993), mitrocomin (Fagan et al., 1993) and obelin (Illarionov et al., 1995).

Table 1: Overview of some photoproteins. The table gives the name, the organism from which the protein has been isolated and the identification number (Acc. No.) of the database entry.

Name	Organism	Identification No.
Obelin	Obelia geniculata	AAL86372
Clytin	Clytia gregaria	CAA49754
Aequorin	Aequorea macrodactyla	AAK02061
Aequorin	Aequorea parva	AAK02060
Mitrocomin	Mitrocoma cellularia	AAA29298
Pholasin	Pholas dactylus	AAM18085
?	Symplectoteuthis oualaniensis	AX305029

Table 2: Overview of some photoproteins. The table gives the organism from which the protein has been isolated, the name of the photoprotein and a selection of patents or applications.

Organism	Fluorescent protein	Patent/Application
Obelia geniculata	Obelin	WO03006497
Aequoria victoria	Aequorin	WO200168824 US-0908909 US 6,152,358 JP-0176125
Pholas dactylus	Pholasin	WO0028025 GB-0024357

Bioluminescence is nowadays used in technology in a wide variety of ways, e.g. in the form of bioindicators of environmental pollution or in biochemistry for sensitively detecting proteins or for quantifying particular compounds, or as what are termed reporters in connection with investigating gene regulation in the cell.

The photoproteins differ not only in their nucleotide and amino acid sequences but also in their biochemical and physical properties.

It has been demonstrated that the physical and biochemical properties of photoproteins can be altered by altering the amino acid sequences of these proteins. Examples of mutagenized

photoproteins are described in the literature (US 6,495,355; US 5,541,309; US 5,093,240; Shimomura et al., 1986).

The abovementioned photoproteins generate light by oxidizing coelenterazine (Haddock et al., 2001; Jones et al., 1999).

Reporter systems

In general, genes whose gene products can be readily detected using simple biochemical or histochemical methods are termed reporter genes or indicator genes. At least 2 types of reporter gene are distinguished.

1. Resistance genes. This is the term used for genes whose expression confers, on a cell, resistance to antibiotics or other substances whose presence in the growth medium leads to the death of the cell if the resistance gene is absent.

2. Reporter genes. The products of reporter genes are used in genetic manipulation as fused or unfused indicators. The commonest reporter genes include beta-galactosidase (Alam et al., 1990), alkaline phosphatase (Yang et al., 1997; Cullen et al., 1992), and luciferases and other photoproteins (Shinomura, 1985; Phillips GN, 1997; Snowdowne et al., 1984).

The emission of photons in the visible spectral range, with this emission being effected by means of excited emitter molecules, is termed luminescence. In contrast to fluorescence, the energy is not, in this case, supplied from the exterior in the form of radiation of shorter wavelength.

A distinction is made between chemiluminescence and bioluminescence. A chemical reaction which leads to an excited molecule which itself luminesces when the excited electrons return to the basal state is termed chemoluminescence. If this reaction is catalyzed by an enzyme, the phenomenon is then referred to as being bioluminescence. The enzymes involved in the reaction are generally termed luciferases.

Classification of the species Bolinopsis infundibulum

Eumetazoa → Radiata → Ctenophora → Tentaculata → Lobata → Bolinopsidae → Bolinopsis infundibulum

Isolating the cDNA

In order to investigate the bioluminescence activity of the species Bolinopsis infundibulum, specimens were caught in the White Sea (Kartesh Biological Station, Russia) and stored in liquid nitrogen. In order to construct the Bolinopsis infundibulum cDNA libraries, the poly(a)+ RNA was isolated using the "Straight A" isolation method from Novagen (USA).

An RT-PCR was carried out for preparing the cDNA. For this, 1 µg of RNA was incubated with reverse transcriptase (Superscript Gold II) in accordance with the following scheme:

PCR	1.	30	seconds	95°C
	2.	6	minutes	68°C
	3.	10	seconds	95°C
	4.	6	minutes	68°C
	17 cycles of step 4 after step 3			

The reaction products were incubated with proteinase K, at 37°C for 30 minutes, in order to inactivate the polymerase, and the cDNA was precipitated with ethanol. The cDNA expression library was constructed using the Clontech (USA) "SMART cDNA" library construction kit in accordance with the manufacturer's instruction. The cDNA was cloned into the expression vector pTriplEx2 (Clontech; USA). The expression vectors were transformed by electroporation into bacteria of the strain E. coli XL1 blue.

The bacteria were plated out on solid LB nutrient medium and incubated at 37°C for 24 hours. A replica plating was then carried out, with the bacteria being transferred to another solid nutrient medium plate using a nitrocellulose filter. The replica plate was in turn incubated at 37°C for 24 hours and the bacterial colonies which had grown were transferred into liquid LB medium. After IPTG (final concentration, 0.1 mM) had been added, the bacteria were incubated at 37°C for 4 hours on a shaker. The bacteria were harvested by centrifugation and the bacterial mass was resuspended, at 0°C, in 0.5 ml of disruption buffer (5 mM EDTA, 20 mM Tris-HCL, pH 9.0). The bacteria were then disrupted by ultrasonication.

After adding coelenterazine (final concentration, 10E-07 M), the lysates were incubated at 4°C for 3 hours. The bioluminescence was then measured in a luminometer after adding calcium chloride (final concentration, 20 mM).

A photoprotein was identified. The photoprotein was designated bolinopsin. The photoprotein bolinopsin is described in detail below.

Bolinopsin

With an identity of 44%, the photoprotein bolinopsin exhibits the highest homology at the amino acid level with aequorin from Aequoria victoria (shown in Example 8; Fig. 7). At the nucleic acid level, the identity is less than 30% (shown in Example 9; Fig. 6). The BLAST method (Altschul et al., 1997) was used for the sequence comparison.

The invention also relates to functional equivalents of bolinopsin. Functional equivalents are those proteins which have comparable physicochemical properties and are at least 70% homologous with SEQ ID NO: 2. Preference is given to a homology of at least 80% or 90%. A homology of at least 95% is particularly preferred.

The photoprotein bolinopsin is suitable for being used as a reporter gene for cellular systems, especially for receptors, for ion channels, for transporters, for transcription factors or for inducible systems.

The photoprotein bolinopsin is suitable for being used as a reporter gene in bacterial and eukaryotic systems, especially in mammalian cells, in bacteria, in yeasts, in baculo and in plants.

The photoprotein bolinopsin is suitable for being used as reporter genes for cellular systems in combination with bioluminescent or chemoluminescent systems, especially systems using luciferases, using oxygenases or using phosphatases.

The photoprotein bolinopsin is suitable for being used as a fusion protein, especially for receptors, for ion channels, for transporters, for transcription factors, for proteinases, for kinases, for phosphodiesterases, for hydrolases, for peptidases, for transferases, for membrane proteins and for glycoproteins.

The photoprotein bolinopsin is suitable for being immobilized, especially by antibodies, by biotin, or by magnetic or magnetizable supports.

The photoprotein bolinopsin is suitable for being used as a protein for energy transfer systems, especially FRET (fluorescence resonance energy transfer), BRET (bioluminescence resonance energy transfer), FET (field effect transistors), FP (fluorescence polarization) and HTRF (homogeneous time-resolved fluorescence) systems.

The photoprotein bolinopsin is suitable for labeling substrates or ligands, especially for proteases, for kinases or for transferases.

The photoprotein bolinopsin is suitable for being expressed in bacterial systems, especially for titer determination, as a substrate for biochemical systems, especially for proteinases and kinases.

The photoprotein bolinopsin is suitable for being used as a label, especially coupled to antibiotics, coupled to enzymes, coupled to receptors or coupled to ion channels and other proteins.

The photoprotein bolinopsin is suitable for being used as a reporter gene in the search for pharmacological active compounds, especially in HTS (high throughput screening).

The photoprotein bolinopsin is suitable for being used as a component of detection systems, especially for ELISA (enzyme-linked immunosorbent assay), for immunohistochemistry, for Western blotting or for confocal microscopy.

The photoprotein bolinopsin is suitable for being used as a label for analyzing interactions, especially for protein-protein interactions, for DNA-protein interactions, for DNA-RNA interactions, for RNA-RNA interactions, or for RNA-protein interactions (DNA: deoxyribonucleic acid; RNA: ribonucleic acid).

The photoprotein bolinopsin is suitable for being used as a label or fusion protein for expression in transgenic organisms, especially in mice, in rats, in hamsters and other mammals, in primates, in fish, in worms or in plants.

The photoprotein bolinopsin is suitable for being used as a label or fusion protein for analyzing embryonic development.

The photoprotein bolinopsin is suitable for being used as a label by way of a coupling mediator, especially by way of biotin, by way of NHS (N-hydroxysulfosuccimide) or by way of CN-Br.

The photoprotein bolinopsin is suitable for being used as a reporter which is coupled to nucleic acids, especially to DNA or RNA.

The photoprotein bolinopsin is suitable for being used as a reporter which is coupled to proteins or peptides.

The photoprotein bolinopsin is suitable for being used as a reporter for measuring intracellular or extracellular calcium concentrations.

The photoprotein bolinopsin is suitable for characterizing signal cascades in cellular systems.

The photoprotein bolinopsin which is coupled to nucleic acids or peptides is suitable for being used as a probe, especially for Northern blots, for Southern blots, for Western blots, for ELISA, for nucleic acid sequencings, for protein analyses or for chip analyses.

The photoprotein bolinopsin is suitable for being used for labeling pharmacological formulations, especially infectious agents, antibodies or "small molecules".

The photoprotein bolinopsin is suitable for being used for geological investigations, especially for ocean, groundwater and river currents.

The photoprotein bolinopsin is suitable for being expressed in expression systems, especially in in-vitro translation systems, in bacterial systems, in yeast systems, in baculo systems, in viral systems and in eukaryotic systems.

The photoprotein bolinopsin is suitable for visualizing tissues or cells in connection with surgical interventions, especially in connection with invasive, in connection with noninvasive and in connection with minimally invasive interventions.

The photoprotein bolinopsin is also suitable for labeling tumor tissues and other phenotypically altered tissues, especially in connection with histological investigation and in connection with surgical interventions.

The invention also relates to the purification of the photoprotein bolinopsin, especially as a wild-type protein, as a fusion protein and as a mutagenized protein.

The invention also relates to the use of the photoprotein bolinopsin in the field of cosmetics, especially bath additives, lotions, soaps, body dyes, toothpaste and body powders.

The invention also relates to the use of the photoprotein bolinopsin for dyeing, especially dyeing foodstuffs, bath additives, ink, textiles and plastics.

The invention also relates to the use of the photoprotein bolinopsin for dyeing paper, especially greetings cards, paper products, wallpapers and handicraft articles.

The invention also relates to the use of the photoprotein bolinopsin for dyeing liquids, especially for water pistols, fountains, beverages and ice.

The invention also relates to the use of the photoprotein bolinopsin for producing toys, especially finger dye and makeup.

The invention relates to nucleic acid molecules which encode the polypeptide which is disclosed by SEQ ID NO: 2.

The invention relates to the polypeptide having the amino acid sequence which is disclosed in SEQ ID NO: 2.

The invention furthermore relates to nucleic acid molecules which are selected from the group consisting of

a) nucleic acid molecules which encode a polypeptide which comprises the amino acid sequence disclosed by SEQ ID NO: 2;

b) nucleic acid molecules which contain the sequence depicted by SEQ ID NO: 1;

c) nucleic acid molecules whose complementary strand hybridizes with a nucleic acid molecule from a) or b) under stringent conditions and which exhibit the biological function of a photoprotein;

d) nucleic acid molecules which differ from the nucleic acid molecules mentioned under c) due to the degeneracy of the genetic code;

e) nucleic acid molecules which exhibit a sequence homology with SEQ ID NO: 1 of at least 95% and whose protein product exhibits the biological function of a photoprotein; and

f) nucleic acid molecules which exhibit a sequence homology with SEQ ID NO: 1 of at least 65% and whose protein product exhibits the biological function of a photoprotein.

The invention also relates to nucleic acid molecules which exhibit a sequence homology with SEQ ID NO: 1 of at least 95%, 90%, 85%, 80%, 75%, 70%, 65% or 60% and which encode a polypeptide which possesses the properties of a photoprotein.

The invention relates to the abovementioned nucleic acid molecules in which the sequence contains a functional promoter 5' to the photoprotein-encoding sequence.

The invention also relates to nucleic acid molecules as previously described which are constituents of recombinant DNA or RNA vectors.

The invention relates to organisms which harbor such a vector.

The invention relates to oligonucleotides having more than 10 consecutive nucleotides which are identical or complementary to the DNA or RNA sequence of the bolinopsin molecules or of the other molecules according to the invention.

The invention relates to photoproteins which are encoded by the previously described nucleotide sequences.

The invention relates to methods for expressing the photoprotein polypeptides according to the invention in bacteria, in eukaryotic cells or in *in-vitro* expression systems.

The invention also relates to methods for purifying/isolating a photoprotein polypeptide according to the invention.

The invention relates to peptides which have more than 5 consecutive amino acids and which are immunologically recognized by antibodies directed against the photoproteins according to the invention.

The invention relates to the use of the photoprotein-encoding nucleic acids according to the invention as marker genes or reporter genes, in particular for searching for pharmacological active compounds and for diagnostics.

The invention relates to the use of the photoproteins according to the invention or of a photoprotein-encoding nucleic acid according to the invention as labels or reporters or as a marker gene or reporter gene.

The invention relates to the use of the photoprotein bolinopsin (SEQ ID NO: 2), or to the use of a nucleic acid which encodes the photoprotein bolinopsin as a label or reporter, or as a label or reporter gene, in particular for searching for pharmacological active compounds and for diagnostics.

The invention relates to the use of the nucleic acid depicted in SEQ ID NO: 1 as a marker gene or reporter gene, in particular for searching for pharmacological active compounds and diagnostics.

The invention also relates to polyclonal or monoclonal antibodies which recognize a polypeptide according to the invention.

The invention also relates to monoclonal or polyclonal antibodies which recognize the photoprotein bolinopsin (SEQ ID NO: 2).

Expressing the photoproteins of the invention

The production of a molecule which, after the gene has been introduced into a suitable host cell, enables the foreign gene which is cloned into an expression vector to be transcribed and translated is termed expression. Expression vectors contain the control signals which are required for expressing genes in prokaryotic or eukaryotic cells.

In principle, expression vectors can be constructed in two different ways. In the case of what are termed transcription fusions, the protein encoded by the cloned-in foreign gene is synthesized as an authentic, biologically active protein. For this purpose, the expression vector carries all the 5' and 3' control signals which are required for the expression.

In the case of what are termed translation fusions, the protein encoded by the cloned-in foreign gene is expressed, together with another protein which can be detected readily, as a hybrid protein. The 5' and 3' control signals which are required for the expression, including the start codon and, possibly, a part of the sequences encoding the N-terminal regions of the hybrid protein to be formed, originate from the vector. The additional protein moiety which is inserted not only in many cases stabilizes the protein, which is encoded by the cloned-in foreign gene, against breakdown by cellular proteases; it can also be used for detecting and isolating the hybrid protein which is formed. The expression can take place either transiently or stably. Suitable host organisms are bacteria, yeasts, viruses or eukaryotic systems.

Purifying the photoproteins of the invention

The isolation of proteins (after they have been overexpressed as well) is frequently termed protein purification. A large number of established methods are available for purifying proteins.

The solid/liquid separation is a basic operation in connection with isolating proteins. This procedural step is required when separating cells from the culture medium, when clarifying the crude extract after having disrupted the cells and removing the cell debris, and when separating off sediments after precipitations, etc. It takes place by means of centrifugation and filtration.

In order to obtain intracellular proteins, the cell wall must be destroyed or rendered permeable. High-pressure homogenizers or agitator ball mills or glass bead mills are used for this purpose, depending on the scale and the organism. Mechanical cell integrations and ultrasonic treatment are used, inter alia, on the laboratory scale.

Both in the case of extracellular proteins and in the case of intracellular proteins (following cell disruption), various precipitation methods using salts (in particular ammonium sulfate) or organic solvents (alcohols or acetone) represent rapid and efficient methods for concentrating proteins. When intracellular proteins are being purified, it is desirable to remove the soluble nucleic acids (precipitation with, for example, streptomycin sulfate or protamine sulfate). When extracellular proteins are being isolated, carriers (e.g. starch or kieselguhr) are frequently added before adding the precipitating agents in order to obtain sediments which are easier to handle.

Numerous chromatographic methods and partition methods (absorption chromatography and ion exchange chromatography, gel filtration, affinity chromatography and electrophoreses) are available for high-degree purification. Column chromatography is also used on an industrial scale. Affinity chromatography, which makes possible purification factors of up to several 100s per step, is especially important for the laboratory scale.

Extracellular proteins accrue in relatively dilute solutions. Just like extracellular proteins, they have to be concentrated before being subjected to further use. In addition to the methods which have already been mentioned, ultrafiltration has proved to be of value, on an industrial scale as well.

Inorganic salts which accompany proteins are frequently undesirable in the case of specific applications. They can be removed by, inter alia, gel filtration, dialysis and diafiltration.

A large number of proteins are used as dry preparations. Important drying methods are vacuum drying, freeze drying and spray drying.

Nucleotide and amino acid sequences

The photoprotein bolinopsin is encoded by the following nucleotide sequence (SEQ ID NO: 1):

5`-
ATGCCTCTAGACGAGACCAACAACGAAAGCTATAGATGGCTGAGAAGTGTGGGTAAC
GATTGGCAGTTTGATGTCGAGGACGTTCATCCTAAACAGCTTAGTCGGCTCTACAAGA
GATTCGACACCTTCGATCTAGACAGTGACGGTCGTATGGACATGGACGAGATCCTGTA
CTGGCCCGACAGAATGAGGCAGCTGGTGAACGCTTCTGACGAACAGGTCGAGAAGAT
GAGGGCTGCTTGCTACACCTTCTTCCACAACAAAGGAGTGGATCCAGAAAAGGGACTC
CTCAGAGACGACTGGGTTGAGGCTAACAGAGTATTTGCTGAGGCTGAAAGAGAGAGG
GAACGACGTGGCATGCCCTCCTTGATTGGTCTTTTGTCAGACGCTTACTACGATGTCCT
GGATGATGACGGTGATGGTACTGTTGATGTTGATGAACTCAAAACCATGATGAAGGCT
TTTGATGTGCCCCAGGAGGCTGCCTACACCTTCTTTAAGAAAGCTGACACGGATAATA
GTGGAAAACTGGAGAGAAGCGAACTGGTCCATCTCTTCAGAAAGTTCTGGATGGAATC
CTACGATCCTCAGTGGGACGGTGTCTACGCTTACAAATATTAA-3`.

This yields an amino acid sequence of (SEQ ID NO: 2):

MPLDETNNESYRWLRSVGNDWQFDVEDVHPKQLSRLYKRFDTFDLDSDGRMDMDEILY
WPDRMRQLVNASDEQVEKMRAACYTFFHNKGVDPEKGLLRDDWVEANRVFAEAERERE
RRGMPSLIGLLSDAYYDVLDDDGDGTVDVDELKTMMKAFDVPQEAAYTFFKKADTDNS
GKLERSELVHLFRKFWMESYDPQWDGVYAYKY

These sequences are reproduced in the sequence listing.

Brief description of the Figures

Fig. 1: Fig. 1 shows the plasmid map of the vector pTriplEX2-bolinopsin.

Fig. 2: Fig. 2 shows the plasmid map of the vector pcDNA3-bolinopsin

Fig. 3: Fig. 3 shows the exitation of bolinopsin. Y: intensity; X: wavelength [nm].

Fig. 4: Fig. 4 shows the fluorescence of bolinopsin. Y: intensity; X: wavelength [nm].

Fig. 5: Fig. 5 shows the bioluminescence of bolinopsin. Y: intensity; X: wavelength [nm].

Fig. 6: Fig. 6 shows the alignment of bolinopsin and aequorin (Aequoria victoria) at the nucleic acid level.

Fig. 7: Fig. 7 shows the alignment of bolinopsin and aequorin (Aequoria victoria) at the amino acid level.

Fig. 8: Fig. 8 shows the result of measuring the bioluminescence of bolinopsin following bacterial expression. Y: luminescence in RLUs [relative light units]; X: µl lysate: 0 = uninduced control lysate.

Fig. 9: Fig. 9 shows the result of measuring the bioluminescence of bolinopsin following bacterial expression in dependence on the coelenterazine derivative employed. Y: luminescence in RLUs [relative light units]; X: coelenterazine derivative: 1 = native, 2 = cp, 3 = f, 4 = fcp, 5 = hcp, 6 = h, 7 = i, 8 = ip, 9 = n; bars: black: uninduced control lysate; light gray: 10 µl of lysate; white: 20 µl of lysate; dark gray: 40 µl of lysate.

Examples

Example 1

The Clontech plasmid pTriplEx2 was used as vector for preparing the construct which is described below. The derivative of the vector was designated pTriplEx2-bolinopsin. The vector pTriplEx2-bolinopsin was used for expressing bolinopsin in bacterial systems.

Fig. 1 shows the plasmid map of the vector pTriplEX2-bolinopsin .

Example 2

The Clontech plasmid pcDNA3.1(+) was used as the vector for preparing the construct which is described below. The derivative of the vector was designated pcDNA3-bolinopsin. The vector pcDNA3-bolinopsin was used for expressing bolinopsin in eukaryotic systems.

Fig. 2 shows the plasmid map of the vector pcDNA3-bolinopsin.

Example 3

Bacterial expression

The bacterial expression was effected in the E. coli strain BL21(DE3) by transforming the bacteria with the expression plasmids pTriplEX2-bolinopsin and pTriplEX2. The transformed bacteria were incubated at 37°C for 3 hours in LB medium and the expression was induced for 4 hours by adding IPTG up to a final concentration of 1 mM. The induced bacteria were harvested by centrifugation, resuspended in PBS + 5 mM EDTA and disrupted by ultrasonication. The lysate was incubated with coelenterazine for 3 hours in the dark. The bioluminescence was measured in a luminometer directly after adding 5 mM calcium chloride. The measurement integration time was 40 seconds.

Fig. 8 shows the results of measuring the bioluminescence of bolinopsin.

Example 4

Eukaryotic expression

Constitutive eukaryotic expression was effected in CHO cells by transfecting the cells with the expression plasmids pcDNA3-bolinopsin and pcDNA3.1(+) in transient experiments. For this, 10 000 cells per well were plated out, in DMEM-F12 medium, on 96-well microtiter plates and the plates were incubated overnight at 37°C. Transfection was effected using the Fugene 6 kits

(Roche) in accordance with the manufacturer's instructions. The transfected cells were incubated overnight in DMEM-F12 medium at 37°C.

Example 5

BLAST

Result of a BLAST analysis of bolinopsin at the amino acid level.

>pdb|1JF2|A Chain A, Crystal Structure Of W92f Obelin Mutant From Obelia Longissima At 1.72 Angstrom Resolution, Length = 195, Score = 85.1 bits (209), Expect = 8e-16, Identities = 52/177 (29%), Positives = 90/177 (50%), Gaps = 4/177 (2%)

>emb|CAD87698.1| unnamed protein product [synthetic construct], Length = 195, Score = 81.6 bits (200), Expect = 8e-15, Identities = 51/177 (28%), Positives = 89/177 (49%), Gaps = 4/177 (2%)

>pdb|1JF0|A Chain A, The Crystal Structure Of Obelin From Obelia Geniculata At 1.82 A Resolution, gb|AAL86372.1|AF394688_1 apoobelin [Obelia geniculata], Length = 195, Score = 80.1 bits (196), Expect = 2e-14, Identities = 51/177 (28%), Positives = 89/177 (49%), Gaps = 4/177 (2%)

>sp|P39047|MYTR_MITCE Mitrocomin precursor, pir||S39022 mitrocomin precursor - Mitrocoma cellularia, gb|AAA29298.1| apomitrocomin, Length = 198, Score = 78.6 bits (192), Expect = 7e-14, Identities = 47/177 (26%), Positives = 91/177 (50%), Gaps = 4/177 (2%)

>sp|Q08121|CLYT_CLYGR Clytin precursor (Phialidin), pir||S28860 clytin - hydromedusa (Clytia gregarium), emb|CAA49754.1| clytin [Clytia gregaria], gb|AAA28293.1| apoclytin, Length = 198, Score = 77.4 bits (189), Expect = 2e-13, Identities = 53/177 (29%), Positives = 89/177 (49%), Gaps = 4/177 (2%)

Example 6

BLAST

Result of a BLAST analysis of bolinopsin at the nucleic acid level.

>gb|AC073341.10| Homo sapiens BAC clone RP11-549I23 from 7, complete sequence, Length = 185574, Score = 52.6 bits (27), Expect = 4e-04, Identities = 33/36 (91%)

>gb|AC092850.13| Homo sapiens 12 BAC RP11-346B9 (Roswell Park Cancer Institute Human BAC Library) complete sequence, Length = 176733, Score = 46.8 bits (24), Expect = 0.023, Identities = 32/36 (88%)

>gb|AC126564.7| Homo sapiens 12 BAC RP11-638F5 (Roswell Park Cancer Institute Human BAC Library) complete sequence, Length = 121242, Score = 46.8 bits (24), Expect = 0.023, Identities = 32/36 (88%)

>gb|AC093924.3| Genomic sequence for Mus musculus, clone RP23-239M9, from chromosome 17, complete sequence, Length = 166277, Score = 44.9 bits (23), Expect = 0.086, Identities = 31/35 (88%)

>gb|AC060234.11| Homo sapiens chromosome 10 clone RP11-523O18, complete sequence, Length = 170073, Score = 44.9 bits (23), Expect = 0.086, Identities = 31/35 (88%)

>gb|AC084727.14| Homo sapiens chromosome 10 clone RP11-507P23, complete sequence, Length = 188652, Score = 44.9 bits (23), Expect = 0.086, Identities = 31/35 (88%)

Example 7

Fig. 6 shows the alignment of bolinopsin with aequorin (Aequoria victoria) at the nucleic acid level.

Example 8

Fig. 7 shows the alignment of bolinopsin with aequorin (Aequoria victoria) at the amino acid level.

Example 9

Spectrum of the photoprotein bolinopsin

In order to measure the spectral properties of bolinopsin, E. coli BL21(DE3) was transformed with the plasmids pTriplEX2-CGFP and pTriplEX2. The induction was effected by adding 1 mM IPTG and incubating at 37°C for 4 hours. The bacteria were then harvested and resuspended in PBS. Lysis was effected by means of ultrasonication. The fluorescence or bioluminescence was then measured. The exitation maximum was at 352 nm, the fluorescence maximum at 452 nm and the bioluminescence maximum at 468 nm.

Fig. 3 shows the exitation of bolinopsin

Fig. 4 shows the fluorescence of bolinopsin

Fig. 5 shows the bioluminescence of bolinopsin

Example 10

In order to identify substrates of bolinopsin, 5 µl solutions of different coelenterazine derivatives (10^{-4} M) in methanol were in each case incubated, at 4°C for 3 hours, with 0, 10, 20 und 40 µl of lysate in a total volume of 75 µl, and the luminescence was measured after adding 25 µl of calcium chloride (final concentration 5 mM). The coelenterazines were obtained from Sigma (Germany). Bolinopsin exhibited bioluminescence activity with all the coelenterazine derivatives employed. The highest activity was measured with the native coelenterazine.

Fig. 9 shows the coelenterazine derivatives as potential substrates for bolinopsin and a graph of the measurement of the luminescence, for 30 seconds at 8.7 kV, in a luminometer (RLUs, relative light units).

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